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GRADED CHANGES IN CENTRAL CHEMOCEPTOR INPUT BY LOCAL TEMPERATURE CHANGES ON THE VENTRAL SURFACE OF MEDULLA

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SUMMARY

1. In cats under pentobarbitone anaesthesia the effects of focal temperature changes of the 'chemoceptive' areas on the ventral surface of medulla, described by Loeschcke and his associates, were studied with respect to tidal volume, V_T, tidal variation in efferent phrenic activity, Phr_T, and respiratory rate. The cats were either paralysed and ventilated at various constant P_{A,CO_2} and P_{a,O_2} levels, or breathing spontaneously.

2. It was confirmed that focal bilateral cooling of the intermediate, '$I_{(S)}$', areas caused rapid depression of respiration even at constant artificial ventilation. In normocapnic and normoxic conditions apnoea usually ensued at brain surface temperatures of 20–22 °C.

3. The effects were graded along continuous temperature–response curves with enhancements of ventilation above and depression below normal body temperature.

4. The strongest effects on V_T and Phr_T were obtained from the $I_{(S)}$ areas with no or only small effects on inspiratory or expiratory timing in the vagotomized animal. The Hering–Breuer inflation reflex and its effects on timing and amplitudes were not affected by cooling this area.

5. Focal cooling of the caudal or the rostral 'chemoceptive' areas, '$C_{(L)}$' and '$R_{(M)}$' areas, caused smaller effects on V_T and Phr_T but produced significant effects on respiratory rate even after vagotomy.

6. The effects of focal cooling of these areas could be mimicked by topical application of procaine solution which has been shown not to penetrate deeper than 100 μm from the surface.

7. Moderate focal cooling of area $I_{(S)}$ to temperatures above 28–30 °C caused a parallel shift in the CO_2–response (V_T, Phr_T) curves to the right with little change in slope. The P_{CO_2} thresholds for apnoea were correspondingly raised. These focal temperature effects could be compensated by changes in P_{CO_2} with, on the average,

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2·7 torr/°C. Focal temperatures below 28 °C usually caused some decrease in slope of the CO_2–response curves in addition to further shifts.

8. Added hypoxic stimulus or electrical stimulation of the carotid sinus nerves caused an almost parallel increase of Phr_T at all P_{CO_2} levels and all focal temperatures suggesting an additive type of interaction between the input from the peripheral chemoreceptors and that from the central (CO_2, H^+) sensing structures whether the latter was altered by changing P_{CO_2} or by focal temperature changes on the $I_{(S)}$ areas.

9. In contrast to these effects of hypoxia and stimulation of the carotid sinus nerves the reflex increase of inspiratory activity caused by lung deflation or by electrical stimulation of the glossopharyngeal nerve distal to the carotid sinus nerves was CO_2 dependent. These reflex effects decreased with focal cooling of the $I_{(S)}$ areas as with hypocapnia, suggesting a mainly multiplicative or 'gain-changing' type of interaction with the central chemoceptive drive.

10. The close similarities in effect of focal cooling and of hypocapnia on the different respiratory parameters even during constant artificial ventilation indicate that focal temperature changes of the $I_{(S)}$ areas intervene effectively with the normal ventilatory response to CO_2 without changing the chemical or physical environment of those neural structures in the brain stem which set respiratory pattern.

INTRODUCTION

In previous papers from this laboratory it was concluded that the stimulating effect on ventilation mediated by the central and peripheral chemoreceptors is mainly projected on to two of the submechanisms involved in the regulation of depth and timing of the breaths, namely (1) the rate of increase of the inspiratory activity in each breath and (2) threshold for inspiratory inhibition leading to inspiratory arrest, i.e. the 'inspiratory off-switch threshold' (Bradley, Euler, Marttila & Roos, 1974*a*, *b*, 1975; Euler, Marttila, Remmers & Trippenbach, 1976; Euler & Trippenbach, 1976). In order to obtain further information on these aspects of ventilatory responses to chemical stimuli attempts were made to study the effects of variations in the input from the postulated central chemoreceptive structures (Åström, 1952; Euler & Söderberg, 1952) to the respiratory neural control mechanisms by means of graded block of this input. This prompted the question whether it would be possible to vary the effects of central chemical stimuli without changing the chemical or physico-chemical environment of the neural networks comprising these control mechanisms.

We have taken advantage of results reported by Loeschcke and his associates who have provided evidence that the central chemoreceptor function is located superficially on the ventral surface of medulla (Mitchell, Loeschcke, Massion & Severinghaus, 1963; Schläfke & Loeschcke, 1967; Loeschcke, de Lattre, Schläfke & Trouth, 1970; Fukuda & Loeschcke, 1977). The Bochum school has further shown that these functions could be reversibly blocked by cooling sites within these superficial chemoceptive areas (e.g. Schläfke & Loeschcke, 1967; Schläfke, See, Massion & Loeschcke, 1969; See, 1976). Thus, after peripheral chemodenervation, complete apnoea was reported to ensue on cooling the 'intermediate' or 'S' area described by Schläfke & Loeschcke (1967) (here designated '$I_{(S)}$' area).

The experiments in this paper examine the effects of focal temperature changes of the superficial 'chemoceptive' areas of the ventral surface of medulla on various respiratory output parameters at different constant levels of P_{A,CO_2}. We have confirmed that focal cooling of the $l_{(S)}$ area rapidly depresses ventilation and found that these effects are graded along a continuous temperature–response curve with enhancement above and depression below normal body tempeature. The results indicate that the two main submechanisms regulating the respiratory pattern were affected in the same way as in response to changes in P_{A,CO_2} although with focal temperature changes the chemical environment of the neuronal elements of these mechanisms was not altered.

The technique of graded depression of the input from the central chemoceptive mechanisms also proved useful in studying the modes of interaction between (CO_2, H^+) and other ventilatory stimuli of reflex or central origin.

A preliminary account of some of our results has been published in brief elsewhere (Cherniack, Euler, Homma & Kao, 1977).

METHODS

Experiments were performed on twenty-three adult cats of either sex (2·2–3·6 kg body weight) anaesthetized with pentobarbitone (Nembutal, Abbott, initial dose 35 mg/kg I.P.). Supplementary doses of pentobarbitone (3–5 mg/kg.hr) and glucose were given by means of a perfusion pump for continuous I.V. infusion.

Surgical preparation commenced by catheterization of a femoral artery and vein and cannulation of the trachea low in the neck. The vagus nerves and carotid sinus nerves were prepared for subsequent section. A phrenic C5 root was dissected free, cut distally and prepared for recording. To gain access to the ventral surface of medulla we have followed the surgical procedures described by Loeschcke *et al.* (1970) and Guertzenstein (1973). Craniotomy of the basal plate of the occipital bone was performed as close to the tympanic bullae as possible. The dura was opened by a mid-line incision and its edges were restracted sideways and fixed by ligatures.

Focal temperature changes were accomplished with a water circulated two-footed thermode, made of lacquered brass. Only the feet of the thermode touched the brain surface with contact areas of 2 by 2 mm. The distance between the feet was 6 mm. The feet of the thermode were slipped under the dura and the lateral bone edges with a slight rotatory movement of the thermode so that the feet came into the desired position on bilaterally symmetrical sites on the surface of medulla. The temperature of the circulating water was adjusted by mixing running hot (about +80 °C) and cold (+5·5 to +6 °C) tap water with the aid of a small reciprocally acting mixing valve close to the thermode. The brain surface temperature under the feet of the thermode was measured either by a small thermistor (0·3 mm in diameter) or a thermocouple probe (0·2 mm diameter).

For topical application of procaine a method was employed similar to that described by Feldberg & Guertzenstein (1972) and Guertzenstein (1973). The procaine solution (5%) was instilled within small O-rings of 3 mm inner diameter to limit the areas subjected to the agent.

Following the routine procedures of this laboratory, mass-phrenic efferent discharge was recorded from the central end of the cut and desheathed C5 root and processed to give a 'moving average' ('integrated' phrenic activity) by a rectifier and RC network containing a third order (Paynter) filter (time constant 100 msec) as described by Evanich, Lopata & Lourenço (1976).

In most cases a large part of the experiment was performed with the animals paralysed with gallamine (Flaxedil, Pharma Rhodia, 4 mg/kg.hr). Artificial ventilation was provided by a 'servo-respirator' (Knox, 1973) which could be governed by the efferent phrenic output so as to hold tracheal pressure closely proportional to the 'moving average' of the efferent phrenic nerve activity. Alternatively the respirator could be controlled at a set rate and stroke volume by the output of an electrical wave generator. The respirator could deliver different gas mixtures from large bags ($\simeq$ 150 l.). Body temperature was kept at 37 ± 0·5 °C by means of a heating pad the power to which is controlled from a rectal thermistor probe.

Tidal volume was measured by electronic integration of the flow signal derived from a differential pressure transducer and a pneumotachograph (Fleisch no. 00). 'Moving average' of phrenic activity, tracheal pressure, arterial blood pressure, rectal temperature, and end-tidal CO_2 concentration were continuously monitored and could be recorded in any selected combinations on a rectilinearly writing four-channel chart recorder and/or displayed on a storage oscilloscope.

RESULTS

Effects of focal temperature changes on the ventral surface of medulla

Generally confirming previous work from the Bochum laboratory (e.g. Schläfke & Loeschcke, 1967; Schläfke *et al.* 1969; See, 1976) we found that focal cooling bilaterally of specific areas of the ventral surface of medulla reduced tidal volume, V_T,

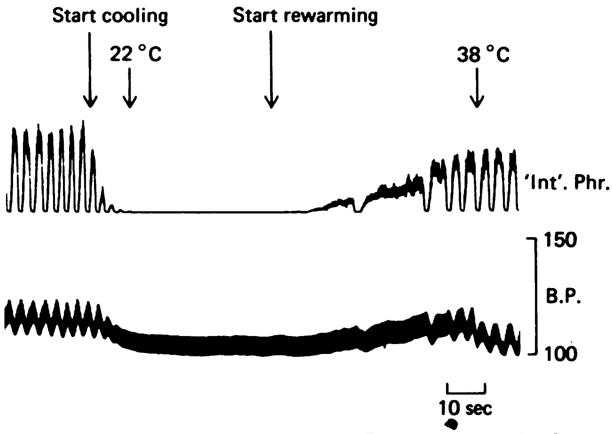

Fig. 1. Effect of focal cooling and rewarming of area $I_{(S)}$ in vagotomized cat on constant artificial ventilation. Upper tracing ('Int.' Phr), moving average of phrenic activity. Lower tracing (B.P. mmHg), blood pressure.

and the tidal amplitude of the 'moving average' of the phrenic inspiratory volleys, Phr_T. The most pronounced responses to cooling in terms of reduction of V_T or Phr_T were obtained when the 'feet' of the thermode were placed bilaterally so as to centre on the $I_{(S)}$ areas located rostramedial to the hypoglossal rootlets. Focal cooling of this area strongly depressed V_T and Phr_T within a few seconds with but small concomitant blood pressure responses as shown in Fig. 1. In 'normocapnic' and 'normoxic' conditions complete apnea was usually obtained at a brain surface temperature of 22–20 °C. In vagotomized cats there were no or only relatively small effects on respiratory rate, or on inspiratory and expiratory durations accompanying the large effects on Phr_T (Fig. 2, cf. also Fig. 7).

Focal cooling of the caudal or L area described by Loeschcke *et al.* (1970) located just medial to the hypoglossal rootlets, hereafter denoted $C_{(L)}$ area, usually provoked somewhat smaller effects on V_T and Phr_T (Fig. 3*C*) and, in addition, an increase in respiratory rate (Fig. 2, lower record). This increase in breathing rate often occurred in steps resembling the response to a rise in body or hypothalamic temperature, except that the step changes to cooling the $C_{(L)}$ area were usually not associated with the appearance of an augmented breath as is usually the case in thermally induced polypnea.

Focal cooling within the rostral or M area between the levels of the 7th and 11th cranial nerves (Mitchell *et al.* 1963) here designated $R_{(M)}$ area, caused no or only small reductions of V_T and Phr_T (Fig. 3*D*). Cooling this area generally produced a reduction in respiratory rate which effect together with the relatively small depression of tidal amplitude caused a marked decrease in the demand for ventilation. The relative effects of cooling the $R_{(M)}$, $I_{(S)}$ and $C_{(L)}$ areas is depicted in Fig. 4*A*.

Outside these areas even long lasting cooling (to 3 min or more) had negligible effects. It was of particular interest that prolonged cooling of the pyramids in positions less than 1 mm medial to areas $I_{(S)}$ and $C_{(L)}$ had almost no effect (Fig. 3*E*).

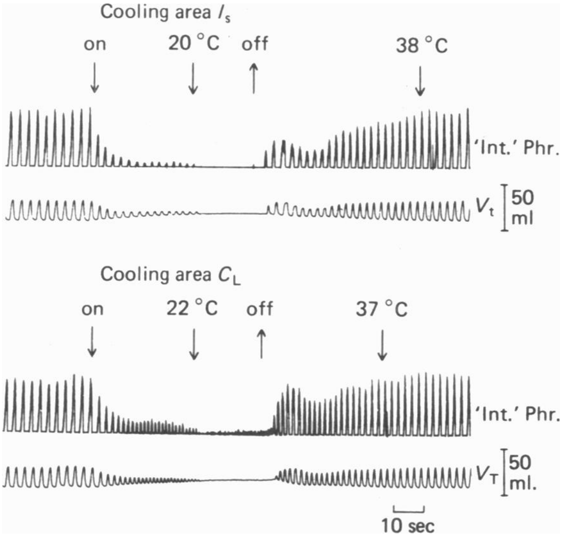

Fig. 2. Effects of focal cooling of $I_{(S)}$ areas (upper pair of records) and $C_{(L)}$ areas (lower pair of records) on tidal phrenic activity ('Int.' Phr.) and tidal volume (V_T). Spontaneously breathing cat after bilateral vagotomy.

Nor was there any significant effect of cooling on the dorsal surface 1–2 mm rostral to obex on sites immediately above the part of the solitarius complex which has a high density of respiratory neurones (see e.g. Euler, Hayward, Marttila & Wyman, 1973) and where lesions have been shown to cause apneustic arrest of respiration (Koepchen, Borchert, Frank, Klüssendorf, Kolbe & Sommer, 1976). This is shown in Fig. 3*F*. The difference in response obtained from different sites are even more striking when the relative depression occurring in the first 10 sec is considered. The time course of the depression to cooling of the $I_{(S)}$ area was considerably faster than at other sites, not only in absolute terms but also proportionate to the final level of depression (see Fig. 3). This suggests that the effect of focal cooling of the $I_{(S)}$ areas is mainly due to cooling of fairly superficial structures.

In the 'open loop' conditions (i.e. in paralysed preparations on constant artificial respiration) unilateral cooling of area $I_{(S)}$ produced a very marked depression (Fig. 3*B*) although usually less than half of that caused by bilateral cooling (Fig. 3*A*).

In spontaneously breathing animals with functional chemostatic feed-back and intact peripheral chemoreceptors, the initial depression of V_T and Phr_T in response to cooling developed much in the same way as in the paralysed cats on constant ventilation. Following the initial depression a subsequent slight increase in V_T and Phr_T occurred in the spontaneously breathing animals probably as a consequence of hypercapnia and hypoxia. This secondary increase could occur even if the initial depression caused apnoea, indicating that even at apnoea the ability of the system to respond to added blood chemical stimuli with reappearance of respiratory activity was retained.

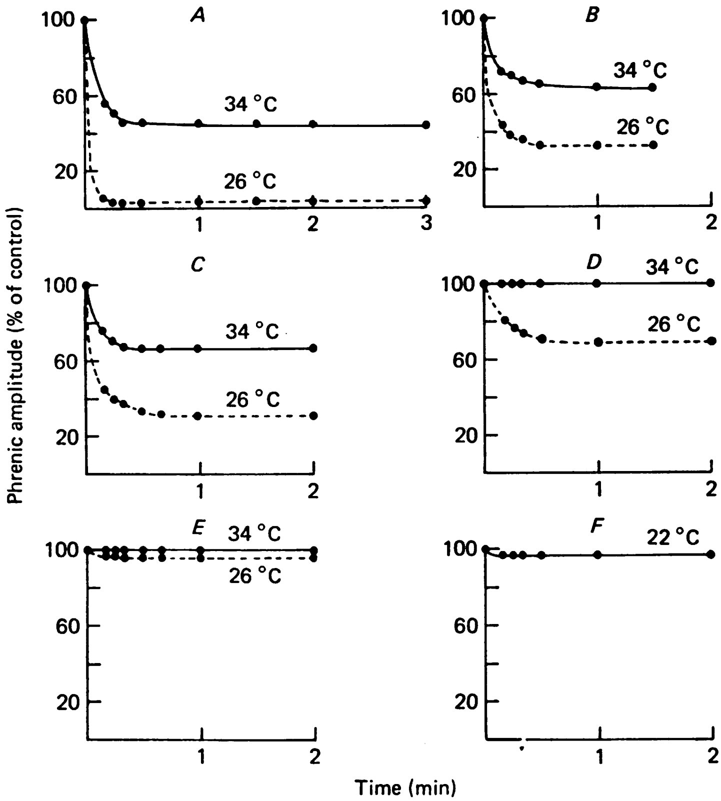

Fig. 3. Magnitude and time course of effects on tidal phrenic amplitude of focal cooling of different sites on the ventral surface of medulla (*A–E*) and on the dorsal surface of medulla (*F*). Vagotomized cats on constant artificial ventilation. Focal cooling was applied: *A* bilaterally on the $I_{(S)}$ areas, *B* unilaterally on one $I_{(S)}$ area, *C* bilaterally on the $C_{(L)}$ areas, *D* bilaterally on the $R_{(M)}$ areas, *E* on the pyramidal tracts with one thermode foot just medial to $I_{(S)}$ and the other foot just medial to $R_{(M)}$ areas, *F* bilaterally on the dorsal surface of medulla with the thermode feet resting bilaterally on sites 2 mm rostral to obex and 3 mm lateral to the mid line.

Effects of topical application of procaine

In order to gain further information concerning the proximity of those structures which are responsible for the effects of focal cooling at the ventral surface we have compared the effects of cooling with the effects of topical administration of procaine. It has been shown that procaine in blocking concentration penetrates only to depths less than 100 μm (Grodinsky, Beber & Baker, 1934; Mitchell *et al.* 1963; Schwanghart, Schröter, Klüssendorf & Koepchen, 1974). Fig. 4*B* shows the effects of bilateral application of procaine on the areas $I_{(S)}$, $C_{(L)}$ and $R_{(M)}$ on Phr_T, respiratory rate, and the product of these two factors ('demand' for ventilation). The effects of procaine on Phr_T were similar to those of focal cooling. However, procaine when applied over the $I_{(S)}$ area sometimes provoked an increase in the respiratory rate. This is probably explained by the fact that the O-rings (3 mm in diameter) were larger than the thermode feet (2 mm) so that part of the $C_{(L)}$ area also became affected by the procaine solution. A pronounced increase in respiratory rate was regularly elicited both

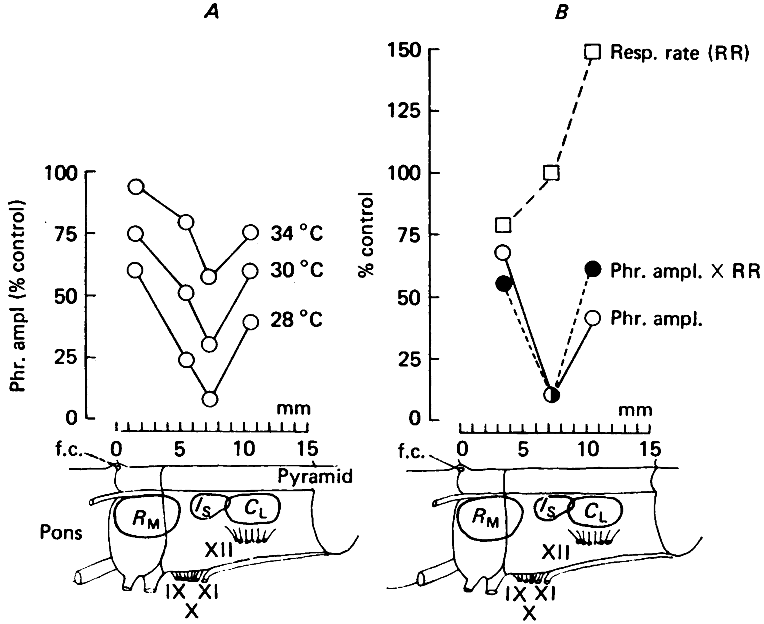

Fig. 4. Effects of focal cooling (*A*) and procaine (*B*) applied on different sites on the ventral surface of medulla in a vagotomized cat on constant artificial respiration. Ordinate: % control values. Abscissa: distance in mm along medullary surface from foramen coecum (f.c.). The relationships between abscissa and the positions of $R_{(M)}$, $I_{(S)}$ and $C_{(L)}$ are shown by the topographical sketches below the graphs.

by focal cooling (see above), and by centring the application of the anaesthetic drug over the $C_{(L)}$ area. In contrast, administration of procaine rostrally on the $R_{(M)}$ area caused a significant reduction in respiratory rate but only small effects on Phr_T. These effects were thus similar to those of cooling this area. Fig. 4*B* shows that due to the decrease in respiratory rate 'demand' for ventilation was reduced more than Phr_T when procaine was applied on the $R_{(M)}$ areas but less than Phr_T when it was applied on the $C_{(L)}$ areas.

Graded temperature effects from area $I_{(S)}$ on V_T and Phr_T

Fig. 5 shows an example of the effects on V_T and Phr_T of changing the temperature of the $I_{(S)}$ areas to different levels. This Figure illustrates that the effects of focal temperature changes did not just occur at a certain 'blocking' level but was a graded, continuous function of the surface temperature between 42 °C and the temperature at which apnoea and phrenic silence occurred. Heating this area to temperatures above normal caused enhancements in V_T and Phr_T continuous with the temperature–response curve obtained below normal body temperatures. The temperature dependence showed Q_{10} values between 3 and 4 over most of the temperature–response curves and different P_{A,CO_2} levels.

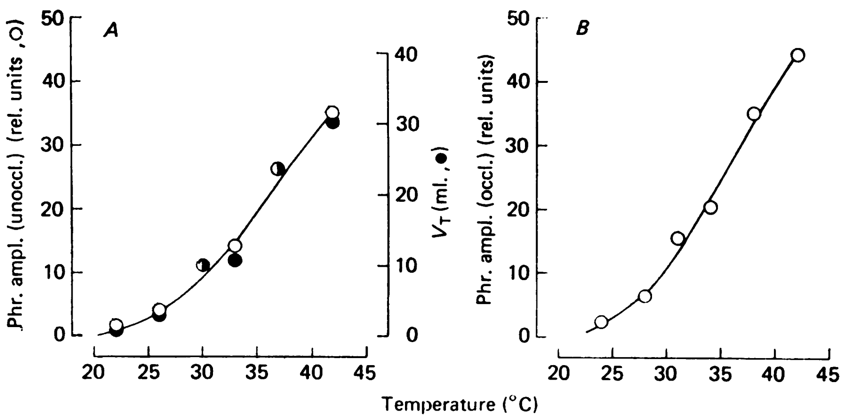

Fig. 5. Effect of focal temperature of the $I_{(S)}$ areas during spontaneous breathing with vagus nerves intact (left panel) and in the absence of cyclic vagal volume related feed-back (right panel). The right-hand graph was obtained after paralysis with gallamine and with the respirator off for single breaths.

In experiments on paralysed cats receiving constant artificial ventilation to eliminate functional chemostatic feed-back control there was no sign of adaptation of the effects when the brain surface temperature was kept constant at different levels. Thus, after the initial rapid changes no further decrease of V_T or Phr_T occurred as cooling was prolonged although the temperature in deeper structures probably continued to fall.

The effect of changing the temperature of the $I_{(S)}$ area was also tested on the apneustic activity which occurred in a few cats on removal of phasic vagal volume feed-back (due to deeper levels of anaesthesia). When area $I_{(S)}$ temperature reached values of 34–30 °C apneusis was usually temporarily broken for a short period of time after which the apneustic activity reappeared. Fig. 6 serves to illustrate that the apneustic level of phrenic activity showed a similar temperature dependence as did Phr_T in the case of regular breathing.

Slope and threshold

With intact as well as with severed vagus nerves focal cooling of the $I_{(S)}$ areas affected phrenic activity by depressing both the amplitude and the rate of rise of the inspiratory activity. In the absence of vagal volume feed-back the former effect suggests a corresponding lowering of the thresholds for inspiratory 'off-switch'. Elevation of the focal $I_{(S)}$ area temperature caused an increase in rate of rise and amplitude of inspiratory activity. Thus these effects resemble the effects of changes in P_{A,CO_2} (Euler & Trippenbach, 1976). In the absence of vagal volume feed-back the relationship between the effects on rate of rise of inspiratory activity and on inspiratory 'off-switch' thresholds, i.e. the degree of 'match' or 'mismatch' (Bradley *et al.* 1974*a*, *b*) is signified by the changes in T_I. The degree of mismatch varied from

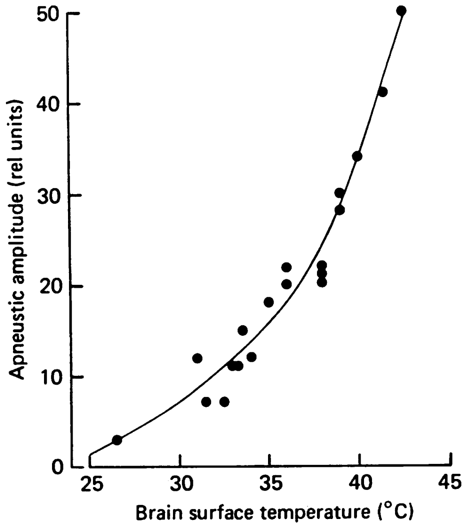

Fig. 6. Effects of focal temperature of the $I_{(S)}$ areas on apneustic activity.

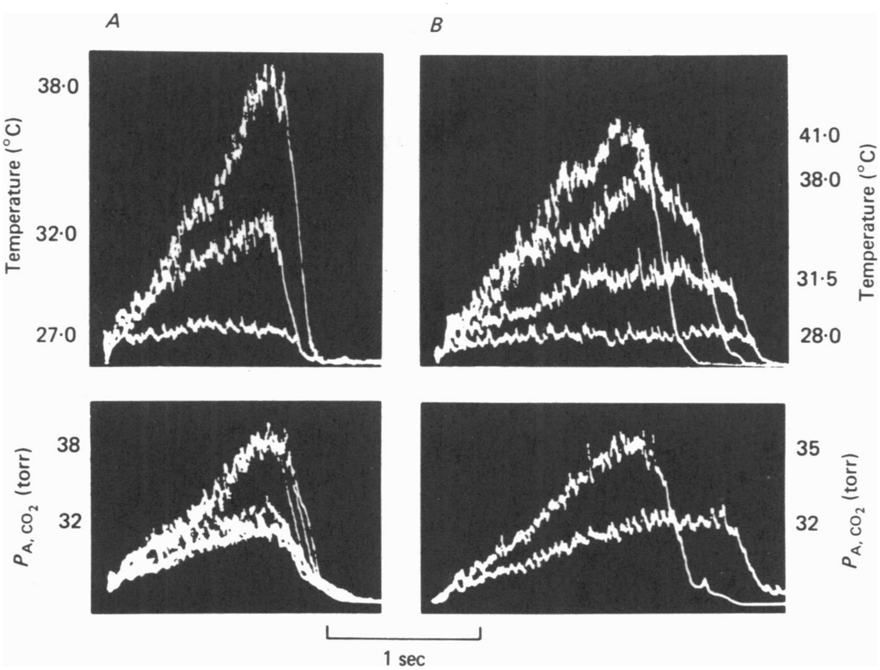

Fig. 7. To compare the effects on rate of increase and duration of inspiratory phrenic activity in response to focal temperature changes on the $I_{(S)}$ areas (upper records) and hypocapnia (lower records) in vagotomized cats. *A* and *B* from two different animals. Superimposed sweeps triggered at the onset of inspiration.

animal to animal and with the level of anaesthesia. In each animal and each experimental condition the degree of mismatch in response to focal temperature changes of the $I_{(S)}$ area was similar to that occurring with changes in P_{A, CO_2}. This is illustrated by the two sets of records in *A* and *B* of Fig. 7 from two different cats. The effects on slope and threshold in response to cooling the $I_{(S)}$ areas are shown in the upper records and the corresponding effects of hypocapnia are shown in the lower records.

Timing

In the vagotomized and paralysed cat focal cooling of the $I_{(S)}$ areas usually caused no or relatively small changes in T_I and T_E accompanying the great changes in amplitude. The effects on T_E were roughly proportional to those on T_I. Cycle duration was unchanged in 60 %, increased in 30 % and decreased in 10 % of the trials. The last mentioned effect, i.e. increase in respiratory rate, did not have any counterpart in a corresponding effect of hypocapnia and might have been due to a slight misplacing of the thermode in caudal direction so as to impinge also upon part of the $C_{(L)}$ area (see above).

On rewarming from apnoea, T_I was often lengthened and sometimes the first inspiratory activity to appear was apneustic in character. Thereafter T_I of the succeeding 'breaths' decreased and breathing activity gradually changed back to the control pattern (cf. Fig. 1). In a few cases a prolongation of T_I occurred just before apnoea was reached in response to cooling or hypocapnia. Similar changes in timing were encountered also on gradual return towards normal P_{A, CO_2} values after the apnoea caused by passive hyperventilation. The presence and the magnitude of the effects on timing in response to focal cooling and hypocapnia showed a considerable amount of variations between individuals. In each animal, however, similar effects were obtained whether apnoea was caused by hyperventilation or by cooling the $I_{(S)}$ areas.

The effect of focal temperature changes of the $I_{(S)}$ areas on the respiratory response to CO_2

Fig. 8 shows a typical example of the effect of changing the surface temperatures of the $I_{(S)}$ areas on the CO_2-dependence of Phr_T. Contrary to our expectations the primary effect of focal temperature changes of the $I_{(S)}$ areas was not to modify the slope of the CO_2-response curves. Instead moderate cooling caused nearly parallel shifts of the relationships between P_{A, CO_2} and the respiratory output parameters towards higher P_{A, CO_2} values (*B* of Fig. 8, see also Figs. 9 and 10). With more intense cooling of the $I_{(S)}$ areas to temperature below 30–28 °C there was usually also some decrease in slope in addition to further shifts of the CO_2–response curves.

The reduction in Phr_T caused by the focal cooling could be compensated for by a sufficient increase in P_{A, CO_2}. Correspondingly, a decrease in phrenic output in response to induced hypocapnia could, within limits, be matched by an increase in temperature of the $I_{(S)}$ areas. This reciprocity between the effects of focal temperature changes and changes in P_{A, CO_2} held true also when Phr_T was reduced to complete apnoea by the focal cooling or by hyperventilation. The shifts in the CO_2–Phr_T curve in response to changes in $I_{(S)}$ area temperature expressed as P_{A, CO_2} per °C show values from 1·0 to 5·2 torr/°C with a mean value in ten cats of 2·7 torr/°C in the temperature range between 38 and 32 °C. Occasionally the slope of the CO_2–response curve flattened as phrenic activity was reduced to near apnoea with hypocapnia. Also in these cases cooling to surface temperature below 28–30 °C caused a further decrease in slope.

The effect of cooling the $I_{(S)}$ areas on the respiratory response to hypoxia and carotid sinus nerve stimulation

Fig. 9 shows the increase in Phr_T in response to hypoxia (P_{a,O_2} = 45 torr) at different P_{A,CO_2} levels and at three different temperatures of the $I_{(S)}$ areas. The upper left panel of this Figure was obtained at a hyperoxic level with P_{a,O_2} values above 200 torr. It shows the CO_2–Phr_T relationship at 38, 32 and 25 °C. At the latter temperatures apnoea was reached at a P_{A,CO_2} of 36 torr. In the other three panels each of these response curves are shown together with the corresponding relationships obtained in hypoxia at a P_{a,O_2} of 45 torr. The effects of a hypoxic stimulation

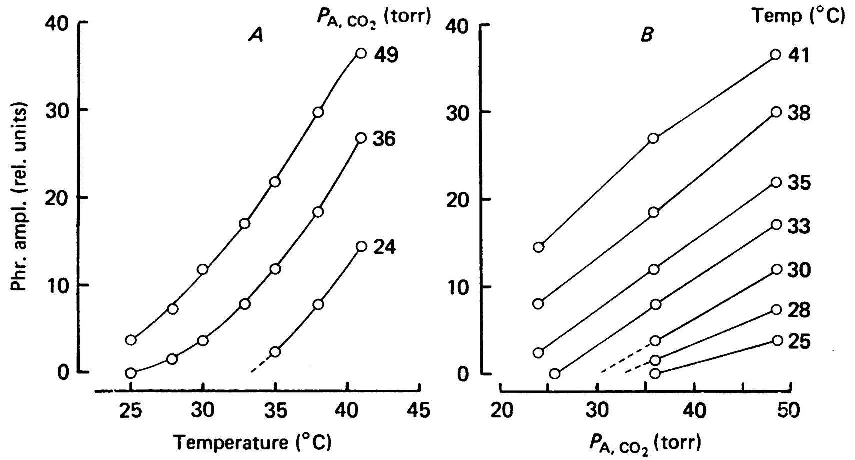

Fig. 8. Effects on tidal phrenic amplitude of $I_{(S)}$ area temperatures at different P_{A,CO_2} levels (left panel) and of P_{A,CO_2} at different $I_{(S)}$ area temperatures (right panel). Same data in left and right panels from a vagotomized cat on constant artificial ventilation.

was to increase Phr_T by roughly an equal amount at the different hypercapnic and hypocapnic levels of P_{A,CO_2} tested. In response to a certain degree of hypoxia the CO_2–response curves thus merely became shifted to higher phrenic amplitudes with little change in slope. This fairly constant amount of additive increase in response to hypoxia was seen at all the different surface temperatures of the $I_{(S)}$ areas tested. However, the shift in response to hypoxia was often smaller at the lower temperatures when the slope of the CO_2–response curve was also decreased. In the temperature range above 28–30 °C the hypoxic response was uninfluenced by changes of $I_{(S)}$ area temperature and the resulting changes in the CO_2–response curves.

The effects of weak and moderate hypoxia on respiratory rate, T_I and T_E, were small in the vagotomized cat. Nor did such hypoxic stimulation in combination with focal temperature changes of the $I_{(S)}$ areas and variations in P_{A,CO_2} cause any more marked effects on timing. Thus the described effects of hypoxic stimulation on Phr_T may be regarded as representative not only for V_T but also for $\dot{V}_E$.

Electrical stimulation of the central end of the carotid sinus nerve had effects similar to hypoxia, i.e. causing a uniform increase of Phr_T at all P_{A,CO_2} levels and at all temperatures tested: no significant change in slope of the CO_2–Phr_T curve occurred, only a parallel shift to higher response values (Fig. 10*A*). When apnoea had been produced by cooling or hyperventilation, or by a combination of the two, electrical stimulation of the carotid sinus nerve proved capable of restoring some tidal phrenic activity and to lower the P_{A,CO_2} necessary to cause apnoea.

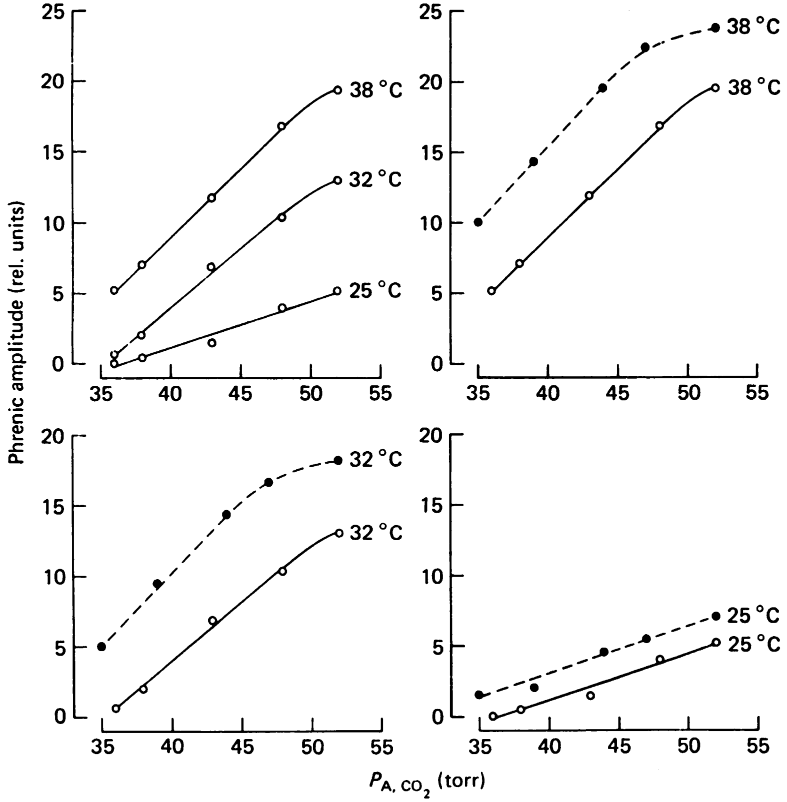

Fig. 9. Effects of hypoxia (P_{a,O_2}: 45 torr, filled circle) on CO_2–response curves at different $I_{(S)}$ area temperatures. Left upper graphs, all at $P_{a,O_2} > 200$ torr, serve as controls. The other three graphs compare control (continuous line and open circles) and hypoxic response curves (dashed lines and filled circles) at each temperature.

The effect of inspiration facilitating reflexes on the CO_2–response at different $I_{(S)}$ area temperatures

Electrical stimulation of glossopharyngeal afferents other than those of the carotid sinus nerve also provoked an increase of the rate of rise and peak amplitude of inspiratory activity. However, in contrast to the mainly additive effects described above for stimulation of the carotid sinus nerve the reflex effects of electrical stimulation of the glossopharyngeal nerve applied distal to the junction of the carotid sinus nerve were dependent on the prevailing inspiratory activity in the unstimulated control condition. With pronounced hypocapnia close to threshold for apnoea only very small reflex effects were obtained but the higher the P_{A,CO_2}, and thus the control inspiratory activity, the stronger the reflex facilitation.

Focal cooling of the $I_{(S)}$ areas had the same effect as hypocapnia on this inspiration facilitating reflex. This is shown in Fig. 10 which compares the effects of electrical stimulation of the carotid sinus nerve (*A*) and of glossopharyngeal afferents other than those of this nerve (*B*) at different P_{A,CO_2} levels and with or without focal cooling of the $I_{(S)}$ areas. Whereas stimulation of the carotid sinus nerve, like hypoxia, caused uniform 'drive'-independent increments of the inspiratory activity, the effects caused by stimulation of the non-carotid sinus nerve afferents of the glossopharyngeal nerve were strongly drive-dependent.

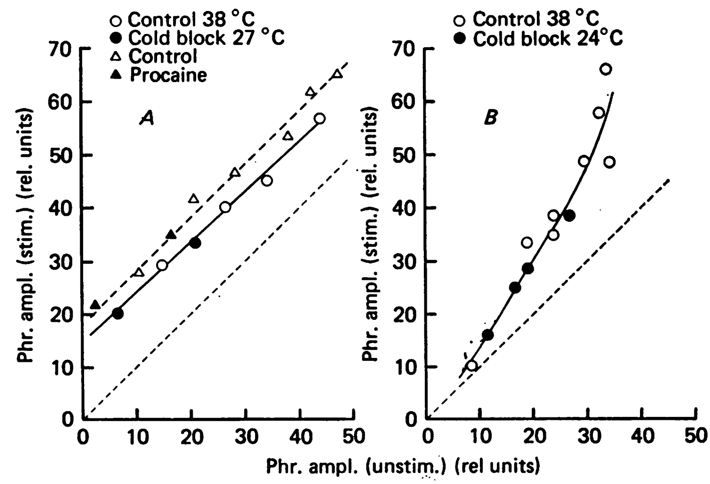

Fig. 10. Effects of electrical stimulation of constant strength of carotid sinus nerve (*A*), and of IX nerve distal to the carotid sinus nerve (*B*) at various P_{A,CO_2} levels before (open symbols) and during (filled symbols) blocking the $I_{(S)}$ areas by focal cooling (circles) and procaine (triangles). Tidal phrenic amplitudes during stimulation (ordinate) are plotted against the unstimulated control amplitudes. Different control amplitudes were obtained by alterations of P_{A,CO_2}. Vagotomized cat at constant levels of artificial ventilation.

Similar drive-dependent facilitatory reflex effects on the inspiratory activity were obtained in response to deflations of the lungs. Fig. 11 illustrates the increase in Phr_T in response to lung deflations of constant magnitude at two different P_{A,CO_2} levels and at six different $I_{(S)}$ area temperatures. The arrangement of this Figure is similar to that of Fig. 9. The upper left panel shows the controls at the different $I_{(S)}$ area temperatures. In the other three panels each of these control response curves are shown together with the curves obtained in the presence of deflations of constant magnitude and at the same temperature. The effect of the deflation reflex was to facilitate inspiration by increasing rate of rise and amplitude of the tidal phrenic activity (Euler, Głogowska & Homma, 1977). This reflex effect was strong in hypercapnia and weak in hypocapnia. As seen in Fig. 11 this caused an increase in slope of the CO_2-response curve at each of the temperatures of the $I_{(S)}$ areas with little effect on the thresholds for apnoea.

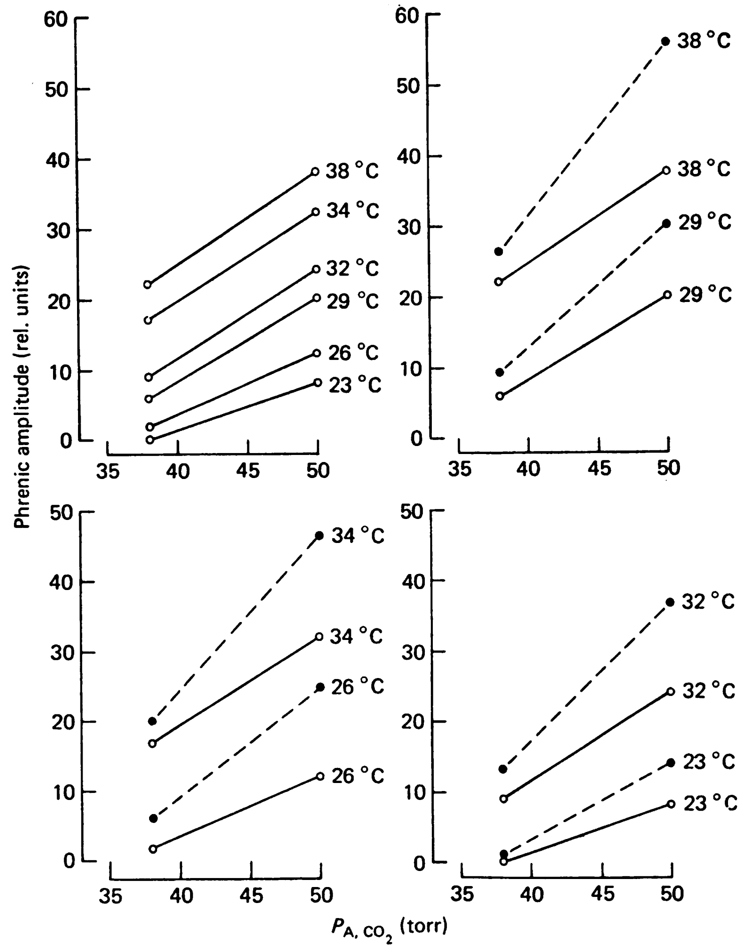

Fig. 11. Effects of lung deflation reflex on CO_2-response curves at different $I_{(S)}$ area temperatures. Left upper graphs, with no deflation, serve as controls. The other three graphs compare control curves (continuous line and open circles) and curves obtained during deflations of 25 ml. below FRC (dashed lines and filled circles). Paralysed cat on constant artificial ventilation. Vagus nerves intact.

No effects of $I_{(S)}$ temperature on Hering–Breuer reflex

The relationships between V_{Teq} (tidal volume at first sign of inspiratory inhibition) and T_I were no different whether the variations in V_T were caused by changing the $I_{(S)}$ area temperature or by changing P_{A,CO_2} as shown in Fig. 12*A*. Nor did focal temperature changes of the $I_{(S)}$ areas cause any detectable changes in the relationship between vagal contribution to inspiratory 'off-switch' and V_{Teq} (Fig. 12*B*).

An approximate indication of the 'vagal contribution' to inspiratory 'off-switch' (Euler & Trippenbach, 1976) was obtained from the difference between Phr_T of breaths with or without cyclic vagal volume feed-back with tracheal cannula open, Phr_C, or occluded at end-expiration, Phr_O (Trippenbach & Milic-Emili, 1977). Part

C of this Figure shows the relationship between 'vagal contribution' and the corresponding effects of 'vagal contribution' on T_I (expressed as T_{I_0} and T_{I_C}) and *D* displays the relationship between V_{Teq} and the effect of 'vagal contribution' on inspiratory timing. None of these relationships were altered by focal cooling of the $I_{(S)}$ areas.

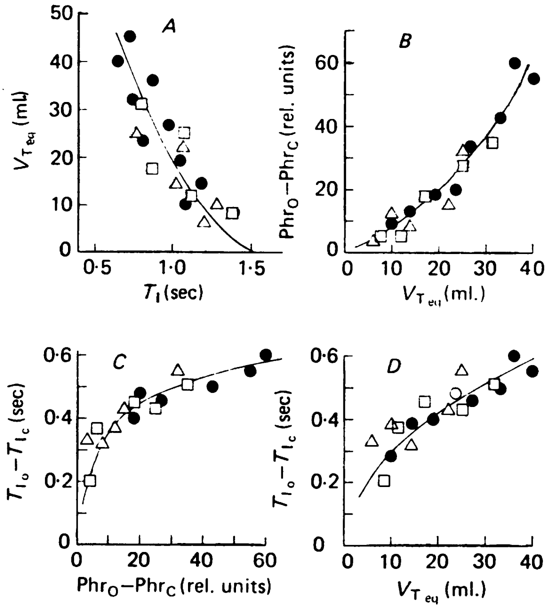

Fig. 12. *A*, vagal volume threshold; *B*, relationship between 'vagal contribution' to inspiratory 'off-switch' and V_{Teq}; *C*, relationship between effect of 'vagal contribution' and its effect on T_I; *D*, relationship between the effects of 'vagal contribution' on T_I and V_{Teq}. The data were obtained at three different $I_{(S)}$ area temperatures. Spontaneously breathing cat with intact vagus nerves. See text. ●, 38 °C P_{A,CO_2} 30–55 torr; □, 32 °C P_{A,CO_2} 38–60 torr; △, 26 °C P_{A,CO_2} 52–70 torr.

DISCUSSION

Structures affected by focal temperature changes

The method of controlled local cooling of sites in the central nervous system for the production of selective block of synaptic transmission with retained, although somewhat slowed, nerve fibre conduction, has been used for many years in the analysis of the functional organization of various neural mechanisms, and the steep spatial temperature gradient in central nervous tissue around a cryogenic probe or thermode (4–10 °C/mm) secures fairly localized effects (e.g. Dondey, Albe-Fessard & Le Beau, 1962; Jasper, Shacter & Montplaisir, 1970; Bénita & Condé, 1972; Brooks, Kozlovskaya, Atkin, Horvath & Uno, 1973).

Our results have shown that within a range of brain surface temperature extending from 42 down to 24 °C there was an almost linear relationship between temperature and tidal phrenic amplitude or tidal volume with Q_{10} values between 3 and 4. In normocapnic animals, apnoea was usually obtained at a surface temperature of 20–22 °C. Since these temperatures are not low enough to cause conduction block even in the most superficial axons (Bénita & Condé, 1972) it may be concluded that the temperature effect reported here, as well as in earlier papers (e.g. Schläfke & Loeschcke, 1967; Schläfke *et al.* 1969; See, 1976), were exerted on structures other than nerve fibres (e.g. membrane and/or synaptic mechanisms) involved in or otherwise affecting the central chemoceptive system for respiratory control.

The temperature dependence of P_{CO_2}, pH and the OH^-/H^+ ratio has been subject to several studies (see e.g. Rahn, 1967; Reeves, 1969, 1977). Precisely how P_{CO_2}, pH and the OH^-/H^+ ratio in the extra- and intracellular compartments change with temperature in the tissue close to a small thermode is a complex matter and not easily understood. Nor do we know how such changes would affect the structures responsible for the effects of the focal cooling described in this paper. Since the effects of focal cooling could largely be imitated by application of procaine it would not seem likely that these effects could be fully explained on the basis of the temperature dependence of the above-mentioned physico-chemical factors.

The effects of focal cooling on the $I_{(S)}$ area differ very much from those of whole body hypothermia. For instance, Pleschka, Albers & Heerd (1965), in experiments on anaesthetized and paralysed dogs, have shown that the CO_2–ventilation curves are shifted to higher ventilation for the same P_{A,CO_2} both when body temperature is raised above and lowered below normal and that both at 40 and at 34 °C the mean P_{A,CO_2} threshold for apnoea was reduced to about 75 % of the mean values for normothermia. The authors concluded that these excitatory effects were not due to direct temperature effects on the bulbar respiratory control mechanisms or the cranial (CO_2, H^+) sensing structures but rather to reflexes from cutaneous receptors. Hypothermia below 30 °C caused progressive depression of respiration and increments of the CO_2 threshold for apnoea to values far beyond those obtained at normothermia. Also the effects of local warming and local cooling of various deeper structures of medulla (apparently also including nucl. retroambigualis) (Holmes, Newman & Wolstencroft, 1960; Chai, Mu & Brobeck, 1965; Chai & Wang, 1970; Chai & Lin, 1972, 1973; Tabatabai, 1972) differ considerably from those obtained in response to moderate temperature changes focally of the $I_{(S)}$ area. Thus, the latter effects cannot be explained on the basis of spread of the temperature changes from the ventral surface to the respiratory neuronal mechanisms located in the depths of medulla either by conduction through the tissue or by convection by the blood.

The close dependence of the temperature effects on the position of the thermode is a further indication that the effects are mediated by structures located close to the surface rather than by deeper respiratory neurones. Strong support for this view is provided also by the similarity in effect of moderate focal cooling and of topical application of procaine which has been shown not to penetrate deeper than 100 μm into the medulla (Grodinsky *et al.* 1934; Mitchell *et al.* 1963; Schwanghart *et al.* 1974). However, the change in slope of the CO_2–response curve observed with cooling below 28 °C was smaller or absent with topical procaine application on area $I_{(S)}$. This difference in effect between focal cooling and application of procaine suggests the possibility that by more intensive cooling other, possibly somewhat more deeply located structures were also affected. In this respect it was important to find that cooling the $I_{(S)}$ area did not influence the Hering-Breuer volume threshold curve and the vagal contribution to 'off-switch' of inspiration. This indicates that in our experiments cooling did not affect the tractus solitarius complex which appears to be of importance for the control of the depth and rate of breathing (Koepchen *et al.* 1976).

Superficial nerve cells have been demonstrated in these areas (Dahlström & Fuxe, 1964; Schläfke, Folgering & Herker, 1973; Trouth, Loeschcke & Berndt, 1973; Dermietzel, 1976). Functionally, the neurones of these areas do not seem to constitute a homogeneous group as can be judged from a variety of investigations (see e.g. Pokorski, 1976; Schläfke, 1976; Fukuda & Loeschcke, 1977; Dahlström & Fuxe, 1964; X. Hökfeldt, personal communication; Feldberg & Guertzenstein, 1976). The apparent functional heterogenity of the superficial neurons might provide explanations for the complex responses obtained when cooling the $C_{(L)}$ area.

Resemblance between focal temperature changes of the $I_{(S)}$ area and systemic changes of P_{CO_2}

An important finding of the present investigation was that in the range above 28–30 °C there was a nearly parallel shift to the right of the CO_2–response curves of 3–5 torr per degree centigrade of focal $I_{(S)}$ temperature with but little change in slope. The CO_2–response threshold, i.e. the apnoea point, also became shifted to higher P_{A,CO_2} values. This suggests that the effects of cooling might be regarded as a de-cruitment, a warming as a re-cruitment of activated elements rather than as changes of the sensitivity of the (CO_2, H^+)-sensing system; sensitivity being defined as the slope of a stimulus–response relationship.

The effects of focal cooling and warming the $I_{(S)}$ areas appeared surprisingly simple in that they closely resembled the effects of changing P_{A,CO_2}. These effects may thus be regarded as being nearly equivalent to changes in input from the intracranial (CO_2, H^+) sensors to the respiratory control mechanisms. This resemblance concerns the effects of P_{A,CO_2} on (1) the rate of rise of inspiratory activity, (2) the 'off-switch' threshold and (3) expiratory timing. Thus the amplitude and the timing aspects of the ventilatory pattern were affected in the same way both when area $I_{(S)}$ was subjected to focal cooling at constant P_{A,CO_2}, and when P_{A,CO_2} was altered by hypocapnia. This implies (1) that by focal cooling of area $I_{(S)}$ we have interfered effectively with the normal ventilatory responsiveness to changes in P_{A,CO_2}, and (2) that the ventilatory effects in response to changes in P_{A,CO_2} are not to any major extent exerted by direct action of (CO_2, H^+) on the neural elements of the rhythm generating and pattern controlling mechanisms but are mediated by a special chemoceptive system (Åström, 1952; Euler & Söderberg, 1952). This system can apparently be effectively influenced at area $I_{(S)}$. It must be emphasized, however, that the present results do not offer any further information as to the actual site of the intracranial receptive structures of this chemoceptive system.

Interaction between central chemoceptive and reflex 'drive'

The close resemblance between the cooling and hyperventilation in decreasing tidal volume and tidal phrenic activity along the same CO_2–response line down to apnoea provides a new method by which to study the interactions between hypoxia, CO_2 and other ventilatory stimuli. The technique might also be useful in the study of the problems of apnoea, and the potential ability for various stimuli singly or in combinations to overcome this condition. Two different types of interactions between CO_2 and other stimuli were observed: (1) a 'multiplicative' or 'gain'-changing interaction and (2) an 'additive' or 'threshold- and recruitment-changing' interaction.

(1) *A 'multiplicative' or 'gain'-changing type of interaction* was observed with deflation reflex and the reflex from glossopharyngeal afferents other than those of the carotid sinus nerve. Above the apnoea point the responsiveness for these reflexes increased with increasing 'drive' and was directly related to the size of the unstimulated control value of Phr_T. One implication of this type of interaction is that it provides some protection against severe hypocapnia by reflex hyperventilation. A similar implication has been suggested for the efferent control of the peripheral chemoreceptors (Majcherczyk & Willshaw, 1973).

(2) *An 'additive' or 'threshold- and recruitment-changing' interaction* was obtained with hypoxia and carotid sinus nerve stimulation. These stimuli caused mainly parallel shifts of the CO_2–response curves to larger responses for isocapnic conditions at all area $I_{(S)}$ temperatures, suggesting a simple arithmetic summation of these stimuli over a fairly large range of input magnitudes. However, hypoxic stimulation sometimes caused a small increase of slope as well as a shift. It might be argued that hypoxic depression could cause a decrease or absence of slope increment with added hypoxic stimulus. However, a change in slope was never seen with electrical stimulation of carotid sinus nerve indicating that the central interaction between afferent CSN input and central chemoceptive stimulation by (CO_2, H^+) is mainly additive in character. In view of the recent results of Majcherczyk & Willshaw (1977) it would seem possible that the increment in slope with hypoxia, when present, might be due to the effect of the efferent, centrifugal control of the arterial chemoreceptors (Sampson & Biscoe, 1970; Neil & O'Reagen, 1971; Majcherczyk & Willshaw, 1973; Trzebski, Zielinski, Majcherczyk, Lipski & Szulczyk, 1974).

With additive interaction the apnoea point, too, is shifted to lower P_{A,CO_2} values. Thus the CO_2–response curves are not only shifted upwards but also extended along the same slope into a P_{A,CO_2} range which was below the normal CO_2 threshold in the 'unstimulated' situation. This means that the intracranial (CO_2, H^+) receptive mechanism has an operational range far below the CO_2 threshold for manifest respiratory output activity. This CO_2 threshold, above which inspiratory activity appears and below which there is apnoea, seems to give room for summation below the threshold of subliminal excitatory inputs from intracranial (CO_2, H^+) chemoceptive structures, arterial chemoreceptors, and from other reflex additively acting sources, e.g. hypothalamic thermoregulatory mechanisms (See, 1976).

Thus modulation of an additively acting ventilatory stimulus might cause effects closely similar to those of focal temperature changes of the $I_{(S)}$ areas. This suggests that the effects of focal cooling might be due merely to a graded reduction of the input of some unknown additively acting respiratory stimulus. Such a possibility presupposes (1) the existence of a strong, tonic ventilatory stimulus present (although possibly somewhat reduced) also in anaesthetized and decerebrate preparations and (2) that this tonic activity relays in the superficial neurones of the $I_{(S)}$ areas. At the present time this possibility has to be taken fully into consideration. The wakefulness drive, for instance, expressed as the differences in CO_2–response curves in wakefulness and 'normal' slow wave sleep appears to exert an additional stimulus of an equal amount of all P_{A,CO_2} levels, i.e. to cause a shift in the CO_2-response curve to the left by 3–5 torr (Reed & Kellogg, 1958, 1960*a*, *b*). In deep slow wave sleep (stage IV), however, a slight decrease in slope in addition to a shift has been reported to occur in dogs, and in some humans a marked decrease in slope was observed (Bülow, 1963). This suggests the possibility that in this state of sleep also some multiplicatively acting inputs might be depressed. The results of the present work indicate, however, that the additively acting input from the peripheral chemoreceptors, or the neural mechanism for its integration with the intracranial (CO_2, H^+) chemoceptive mechanism, is not affected by moderate cooling of the $I_{(S)}$ areas.

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